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CANADA
DEPARTMENT OF AGRICULTURE
DOMINION EXPERIMENTAL FARMS

APICULTURE DIVISION

DOMINION EXPERIMENTAL FARM, OTTAWA, CANADA

C. A. Jamieson, B.S.A., Ph. D., Chief

PROGRESS REPORT 1949-1953



Honeybees collecting nectar and pollen from typical red clover blooms.
(See inside front cover.)

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COVER PICTURE—Two honeybees are shown collecting nectar and pollen from typical red clover blooms. Danish, German, English, New Zealand, American, and Canadian research workers have shown that honeybees are efficient pollinators of red clover. The corolla tube length varies from 8 to 9.6 mm. The honeybee tongue lengths range from 5.8 to 6.4 mm. The honeybee can extend its head one mm. into the floret thus procuring the nectar when it reaches a height of 2 mm. in the floret. The height of the nectar column may, on occasion, reach 5 mm. or more.

The concentration of nectar varies from 17 to 60.6 per cent total solids, and is attractive to honeybees when it rises above 40 per cent.

The honeybees will visit the red clover to gather pollen, particularly when that of competing crops is not available. Pollen collections showed that colony preferences for red clover ranged from 11 to 71 per cent, depending upon the competing flora in bloom in the area at the same time.

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INTRODUCTION

During the five-year period (1949 to 1953) covered in this report, increased use has been made of honeybees for the specific purpose of the cross-pollination of legumes, fruits, and vegetables. Many legume seed producers are requesting beekeepers to establish apiaries on their farms. In some cases a rental fee is paid but for the most part the grower offers only a free apiary location for the pollination service. Several thousands of colonies are rented each year in Ontario, Quebec, and British Columbia for fruit pollination.

The increased use of bees for pollination, however, has not stimulated a greater interest in beekeeping. The data presented in Table 1 show the changes in the magnitude of the industry during the past five years.

TABLE 1.—NUMBER OF BEEKEEPERS, COLONIES, AND PRODUCTION IN CANADA^a
1949 to 1953^b

	Beekeepers	Colonies	Total Honey Production
	No.	No.	'000 lb.
1949.....	25,870	473,450	31,481
1950.....	22,180	430,000	28,351
1951.....	18,900	406,300	40,909
1952.....	15,950	385,600	31,230
1953.....	13,950	341,300	26,384

^a Data for Newfoundland not available.

^b Data from Report of Bureau of Statistics.

The number of colonies and number of beekeepers have been considerably reduced in all provinces during the past five years. Unfavorable prices are largely responsible for the decline in interest in beekeeping. On the other hand most commercial beekeepers have had to increase the number of colonies operated to offset lower returns. At the close of this period honey prices generally showed a marked improvement. Beekeeping expanded in the Peace River district in 1953 and a further expansion in this area is expected. There are large acreages of such legumes as sweet clover, alfalfa, and alsike clover available for bee pasture in the British Columbia and Alberta districts of the Peace River country.

With the exception of 1950 and 1953 the total average honey production in Canada has been maintained even though there are fewer colonies. Adverse weather conditions during the honey-flow period reduced the crop in those years.

Since the publication of the last Progress Report (1948) there has been expansion in several phases of work. Increased emphasis has been placed on investigations of the value of honeybees for pollination. An extensive project has been undertaken to determine the value of honeybees in tripping and cross-pollinating alfalfa at the Regina Substation. Pollination studies at Ottawa have been mainly concerned with the factors affecting production in red clover. Fundamental studies on the crystallization behavior of honey have been initiated to provide a clearer understanding of the factors concerned in quality packing of liquid and recrystallized honey. A study of the viscosity and flow rates of honey has also provided basic information for beekeepers and those engaged in packing honey. Two types of equipment for processing honey have been investigated. A unit of large capacity and a smaller unit designed for the beekeeper-packer have been tested.

Many beekeepers in Canada have adopted chemo-therapeutic treatments of colonies to control the spread of brood diseases. Investigations on the use of sulphur drugs and antibiotics for this purpose are continuing at Ottawa and Brandon. New methods of control of *Nosema* disease in adult honeybees by antibiotics were initiated at Ottawa.

Several staff changes have taken place since the issue of the last Progress Report. C. B. Gooderham, former Chief of the Division, retired in 1949. C. A. Jamieson, previously Assistant at the Division was appointed Chief in the fall of 1949. J. H. Arnott resigned from the Division in 1949 to accept the position of Provincial Apiarist in Saskatchewan. E. Braun, formerly in charge of the apiary work at Brandon, was transferred to Ottawa as Assistant in 1950. G. H. Austin and P. Pankiw joined the staff in 1950 and 1951, respectively.

A new Branch Farm Apiary was established in 1951 at the Regina Substation to study the effectiveness of honeybees as pollinating agents of alfalfa. This project, under the direction of P. Pankiw, was concluded in the fall of 1953 and the equipment transferred to Beaverlodge, Alta. At this station work will be undertaken on the factors affecting seed production of alsike clover as well as problems concerned with the management of bees and control of disease.

POLLINATION STUDIES¹

Seed production in many of the leguminous plants is dependent upon insects for cross-pollination. Fluctuation in numbers of native pollinators in seed-producing areas makes seed production a hazardous venture. Honeybee populations, which can be controlled, may be used to good advantage for the pollination of numerous crops. Some of the factors affecting the foraging behavior of honeybees were investigated on red clover and alfalfa during the years 1949 to 1953.

The following work was a co-operative project between the Apiculture and Forage Crops Divisions, Experimental Farms Service and the Entomology Division, Science Service.

Red Clover

Insect Survey

The presence of injurious and beneficial insects was determined prior to the first cut and also prior to and during the second bloom of red clover.

The insect complex, determined by the Entomology Division, consisted of the following: tarnished plant bug (nymphs and adults); six-spotted leafhopper; meadow spittlebug; *Plagiognathus chrysanthemi* (Wolff); pea aphid; potato leafhopper; red-headed flea beetle; clover root curculio; lesser clover leaf weevil; clover head weevil; clover seed midge; and grasshoppers.

Partial control of these injurious insects, with the exception of the clover seed midge, was secured by the use of toxaphene and DDT applied at various times.

The pollinating insects were limited to the following: *Bombus*, *Andrena*, *Megachile*; *Halictus*, *Lassioglossum*, *Heriades*, and *Certina* on the first bloom. Honeybees and bumblebees were considered the primary pollinators on the second bloom and will be dealt with under "pollinator surveys".

Pollinator Surveys

(a) *Pollinator Counts*.—Foragers were counted on two-square-yard counting stations spaced at regular intervals through the fields. The counts were made at hourly intervals each day during the experimental period.

¹ E. Braun.

Pollinator activity varied widely from day to day and was significantly higher in the afternoon than in the morning. The population density of honeybees and bumblebees on red clover fields was determined for three seasons as outlined in Table 2.

TABLE 2.—POPULATION DENSITY OF HONEYBEES AND BUMBLEBEES AND SEED YIELDS ON FIELDS OF RED CLOVER FOR THE YEARS 1950, 1951, AND 1952

Year	Location and Farm	Number of colonies of bees	Pollinators present based on a 10-square-yard area (mean of all timed observations)		Mean seed yield per acre in pounds
			Honeybees	Bumblebees	
1950	Carp..... Wilson.....	1	125.0	14.7	143
1951	Carp..... Wilson.....	2	32.8	14.9	25*
1952	Carp..... Bracken (10).....	1	10.5	4.1	261
	Carp..... Bracken (30).....	1	2.0	2.1	123
	Ottawa..... Croskery.....	1	8.7	2.5	102
	Ottawa..... Scott.....	1	16.0	0.5	336
	Renfrew..... Johnston.....	0	1.1	3.8	63
	Renfrew..... Forrest.....	1	2.0	5.4	125
	Renfrew..... S. Barr.....	2	6.5	4.6	307
	Renfrew..... D. Barr.....	3	15.2	4.5	233
Renfrew..... Kluge.....	5	8.8	4.5	283	

* The seed yield in 1951 was reduced because of a severe clover seed midge infestation.

The data show a wide variation in population density of honeybees between different seasons and between fields in different areas during the same season. Adjacent to the Kluge field at Renfrew there was a 30-acre field of buckwheat which attracted the foragers from the red clover. Populations decreased in this field when the buckwheat began to yield nectar. Bumblebee populations varied between seasons but were relatively constant in any single field. The low population on the Scott field was due to its location in an urban area with limited nesting sites for bumblebees in the surrounding fields.

Seed yields varied considerably depending upon the type of stand, date of first cut for hay, insect damage, and other factors. Although no apparent correlation is evident between bumblebees and seed yield some relationship between honeybees and seed yield in fields in the same area is indicated. Further tests are required to verify such a conclusion.

(b) *Visitation Rates of Honeybees and Bumblebees (1949).*—The number of florets visited during a two-minute period showed that 25 honeybees visited an average of 26.3 florets compared with 60.9 florets visited by the bumblebees. The following species of bumblebees were identified from the red clover field: *Bombus fervidus* (Fabr.); *Bombus borealis* (Kby.); *Bombus vagans* (F. Smith); and *Bombus pennsylvanicus* DeG.

(c) *Pollinator Training.*—A dilute solution of honey was sprayed on randomized plots of red clover to attract pollinators but this did not increase the seed yield.

(d) *Colony Placement.*—The importance of colony placement was indicated by the fact that (a) the number of honeybee pollinators decreased as the distance from the apiary increased, (b) the greater the number of honeybee pollinators the greater the seed yield, and (c) seed yield decreased as the distance from the apiary increased.

Nectar Investigations

The volume and concentration of nectar varied significantly between days. During the day the volume was significantly higher in the morning and concentration was higher in the afternoon.

Plots sprayed with DDT, toxaphene and a combination of both materials showed significantly higher concentration than unsprayed plots but there was no significant difference in the volume of nectar between these plots.

Floral Studies

The volume of nectar in fertilized and unfertilized flowers was found to diminish very rapidly after the fifth and seventh day, respectively. The third day after fertilization the percentage of total solids decreased, but this was not apparent in unfertilized flowers until seven days after full bloom.

Pollen Collections

Analyses of pollen collected from pollen traps indicated a dispersal of some pollen gatherers from the crop to be pollinated to competing crops.

TABLE 3.—RED CLOVER POLLEN IN RELATION TO COMPETING CROPS

Year	Location	Farm	Percentage of pollen collected by honeybee colonies	
			Red clover pollen	Other types of pollen
1951	Carp.....	Wilson.....	42.0	58.0
1952	Carp.....	Bracken.....	30.0	70.0
	Ottawa.....	Croskery.....	55.0	45.0
	Ottawa.....	Scott.....	32.0	68.0

As indicated in Table 3 there was a great variation in the amounts and kinds of pollen gathered by individual colonies. Red clover pollen collections ranged from 11 to 71 per cent. On the basis of all pollen gathered that of red clover was third in order of importance, being 24 per cent of the total weight. Day to day comparisons showed that the amount of this pollen collected increased from 4 to 83 per cent from the first to the last day of the test.

Seed Yields

Pollinator population, colony placement and distance of flight have been shown to have a direct influence on the seed yield of red clover. A marked reduction of both pollinator population and seed yield was observed when the distance the bees had to travel exceeded 500 yards. Seed yields ranged from 63.3 pounds per acre on the control field, pollinated by native pollinators and a few honeybees, to 307 pounds when two colonies of honeybees per acre were used. Another field averaged 336 pounds per acre when only one colony per acre was used. The number of colonies necessary for adequate pollination will depend upon the competing crops in any area and their relative attractiveness to honeybees.

Effect of Insecticide Treatments on Seed Yields of Red Clover

When randomized plots of red clover (1950) were sprayed at the pre-bloom stage with DDT, and with toxaphene at full bloom the seed yield was increased by as much as 25 per cent. Toxaphene provided an excellent control of grasshopper infestation on the treated plots.

Spray treatments were applied to plots in a field of red clover in 1951 but there was no significant difference in the seed yields between the treated and check plots. The spray materials were not effective in controlling a heavy infestation of the red clover seed midge. Approximately 70 per cent of red clover heads were destroyed or damaged by this insect.

Further spray and dust treatments were applied to fields of red clover in 1952. While a good measure of control was obtained of several insect pests there was no significant difference in seed yields between the treated and control plots.



FIG. 1.—Pollinator-counting plot on red clover (1949).

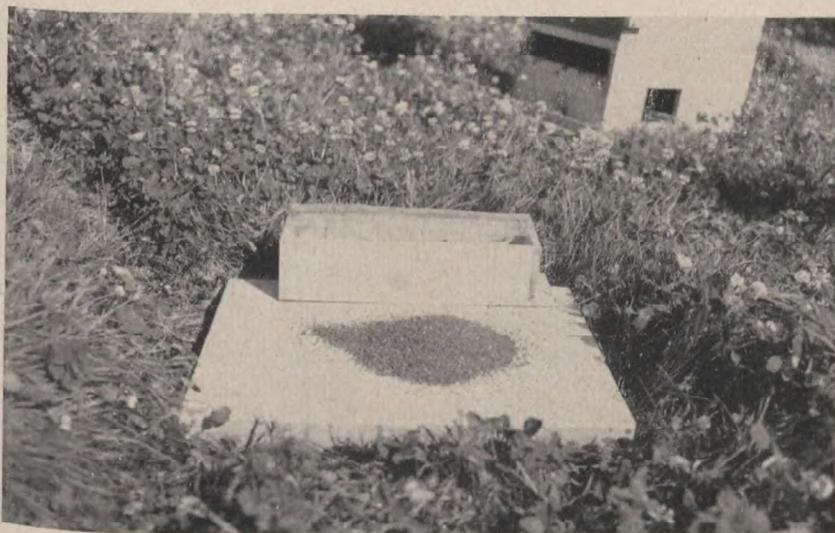


FIG. 2.—Pollen collection from trap.

Observations on the Pollinating Insects on Alfalfa

The number, kinds and behavior of bees visiting plots (two square yards in area) were recorded in a small block of alfalfa in 1949. An apiary of 125 colonies of honeybees was located within one-half mile of the field of alfalfa. During a six-day period 1,642 honeybees and 456 native bees were observed on the plots. None of the honeybees were seen to trip the florets, but were collecting nectar from the side of the petals. Andrenids, Halictids, and Megachilids

were present with the last mentioned being the most consistent tripper on the florets. A few bumblebees were also observed but they were not tripping the florets. Three pollen-gathering honeybees, outside of the observation plots, were collected and the pollen identified as that of alfalfa.

In 1952 two honeybee colonies per acre were placed on an alfalfa field and the ratio of honeybees to bumblebees was 66: 1 per two-square-yard counting area for a period of thirteen days. The bumblebees remained fairly constant over the field but the honeybees decreased as the distance from the hive increased. The percentage of florets tripped per raceme was an average of 2.31 over a four-day period. The tripping rate was higher in the afternoon than in the morning. No observations were made on the tripping rate of different bees.

An alfalfa varietal study in 1953 showed a three-day average of 16.6 honeybees, 0.003 bumblebees and 0.37 wild pollinators per two-square-yard area on Grimm alfalfa and slightly less on the M-50 variety. The bloom data showed an average of 39 racemes per plot for M-50 as compared with 31 for the Grimm variety, while the range of florets per raceme was identical, between 9 and 20 on both varieties. A daily average of 5.46 per cent of all florets observed were tripped by honeybees, with the rate being slightly higher for the Grimm than the M-50. One honeybee was observed to visit 14 florets and to trip 12 of them.

The tripping rate for the wild pollinators averaged between 87 and 91 per cent but the numbers of wild pollinators present were limited compared with the honeybees. The concentration of nectar, based on percentage of total solids, was similar for both varieties.

The average seed yield obtained was 56 pounds per acre from M-50, and 33 pounds from the Grimm variety. Selfing is similar in both varieties and constitutes about one-third of the seed set which was obtained by cross-pollination. This indicates that cross-pollination is essential for both of these varieties.

Toxicity of Insecticides to Honeybees on Legumes¹

This was a co-operative project between the Apiculture and Forage Crops Divisions, Experimental Farms Service, and was conducted in conjunction with the previous work on pollination.

DDT, toxaphene and methoxychlor, either as spray or dust, were applied to the crops in four treatments and a check on the basis of five replications during 1950, 1951, and 1952.

1. DDT applied just previous to bloom.
2. DDT applied just previous to bloom and toxaphene at full bloom.
3. DDT applied just previous to bloom and methoxychlor at full bloom.
4. DDT plus toxaphene applied just prior to bloom.
5. Check (control).

Application

Chemicals were applied as sprays, at a pressure of 225 p.s.i. (pounds per square inch) or 40 p.s.i., in approximately 50 gallons of water, per acre. Rate of application was as follows:

DDT—25 per cent emulsion—1.5 pounds active ingredient per acre.

Toxaphene—50 per cent emulsion—1.5 pounds active ingredient per acre.

Methoxychlor—50 per cent wettable powder—1.5 pounds active ingredient per acre.

The dusts were applied at the rate of 2 pounds per acre at the pre-bloom stage.

¹ E. Braun.

Observations on Toxicity

Traps were placed at the entrances of five colonies and collections of dead bees were made daily. Collections from other colonies not subject to the insecticides were used as controls. No significant difference was observed between colonies on treated or untreated fields when either sprays or dusts were used. The mortality rate was higher on the sprayed than on the dusted areas.

Colony strengths were determined prior to placing them in the fields and periodic examinations, while in the field, did not reveal any abnormal loss from the use of the insecticides.

Similar observations were made on alfalfa and birdsfoot trefoil. Additional fields belonging to farmers were also treated to determine the effect on the seed yields.

Honeybees and Orchard Sprays¹

In 1948 some data were obtained on the toxicity of parathion to honeybees under field conditions. Further observations were made in 1949 on this insecticide.

Two strong colonies of bees, equipped with specially constructed cages for trapping sick and dying bees, were moved into an orchard on May 9. On May 13 a parathion spray was applied at the rate of 1 pound of 15 per cent wettable powder per 100 gallons. When this spray was applied there were six trees in full bloom and six other trees showing partial bloom. On May 16 a spray was applied at the rate of one-half pound of 25 per cent wettable powder per 100 gallons to the remainder of approximately 100 trees in full bloom. Daily counts were made of the dead bees in the cages.

TABLE 4.—DEAD BEES COLLECTED IN CAGES BEFORE AND AFTER TREES WERE SPRAYED WITH PARATHION

Date	Number of bees	Average dead 3-day period	Remarks
May 10.....	120	127	Bees mostly inactive
May 11.....	107		" " "
May 12.....	64		" " "
May 13*.....	189	242	Bees working
May 14.....	350		" "
May 15.....	188		" "
May 16*.....	1,555	709	" "
May 17.....	487		" "
May 18.....	80		" "
May 19.....	35		" "

* Dates on which sprays applied.

It is evident from the data shown in Table 4 that an increase in mortality occurred following application of spray. The highest mortality occurred on May 16 when parathion was applied to the larger number of trees in full bloom.

A sample of dead bees was analysed for parathion and found to contain 0.13 micrograms of the insecticide per bee. The LD₅₀ for parathion has been established at 0.07 micrograms per bee.

¹ C. A. Jamieson.

Effect of Herbicides on Honeybees (1952)¹

A preliminary test was conducted to ascertain the relative effect of different concentrations of sugar on the longevity of honeybees. It was found that a 10 per cent sugar solution was inadequate to sustain life in a honeybee. The most satisfactory sucrose solutions were 40, 50 and 60 per cent.

2,4-D:

The lethal dose of the 2,4-D water-soluble salts, in varying solutions of sugar syrup was determined. The amount of 2,4-D consumed was calculated from the weight of the feed taken up by the bees. The results are shown in Table 5.

TABLE 5.—MORTALITY PERCENTAGES BY DAYS AND LETHAL DOSES OF .1 PER CENT 2,4-D ACID IN VARYING SUGAR CONCENTRATIONS

Percentage sugar solution	Days						Number of bees, two replicates	Lethal dose micrograms
	1	2	3	4	5	6		
50.....	5	37	56	90	99	100*	282	64
40.....	4	67	87	94	100*		288	67
30.....	4	56	82	97	100*		287	67
20.....	0	78	98	100*			260	64
10.....	14	86	100*				290	65

* A few bees were still moving, but feebly, and were not able to take up any more food.

The data show that the mortality rate increased as the concentration of syrup decreased because the bees had to ingest more of the syrup to satisfy their food requirements and consequently ingested more of the herbicides. The lethal dose was similar for the different groups of solutions used.

The following is a list of the other herbicides tested and for which lethal dosages were established: Isopropyl amine 2,4-D; Tri-ethanol amine 2,4-D; Sodium salt 2,4-D; Butoxy ethanol ester 2,4-D; Methoxone butyl ester 2,4-D; Isopropyl ester 2, 4-D; Isopropyl phenyl-N-carbamate; Maleic hydrazide; and 3-P-chlorophenyl-1-1-dimethylurea (C.M.U.). Calculated on a unit weight basis, Maleic hydrazide was the least toxic of the herbicides followed by C.M.U. and lastly the 2,4-D salts.

The physiological reactions of bees fed 2,4-D are excitability, paralysis of the wings and legs, finally reduced movements and death. This may take from a few hours to several days.

Some Factors in the Breakdown in Texture of Recrystallized Honey¹

In packing recrystallized honey, quality deterioration as manifested by a softening of crystal texture is frequently encountered. When this softening proceeds to the extent that separation into two phases occurs, the honey should be reprocessed or sold at a lower price.

An investigation was undertaken to evaluate the factors concerned in this breakdown. The two factors most concerned are the moisture content and the

¹ P. Pankiw.

ratio of levulose to dextrose in the honey. An instrument (penetrometer) used in the petroleum industry to measure the consistency of heavy greases was slightly modified so that changes in the firmness of honey texture could be determined. With the penetrometer (Fig. 3) it is possible to measure the depth in tenths of millimetres that a weighted needle penetrates a substance in a given time.

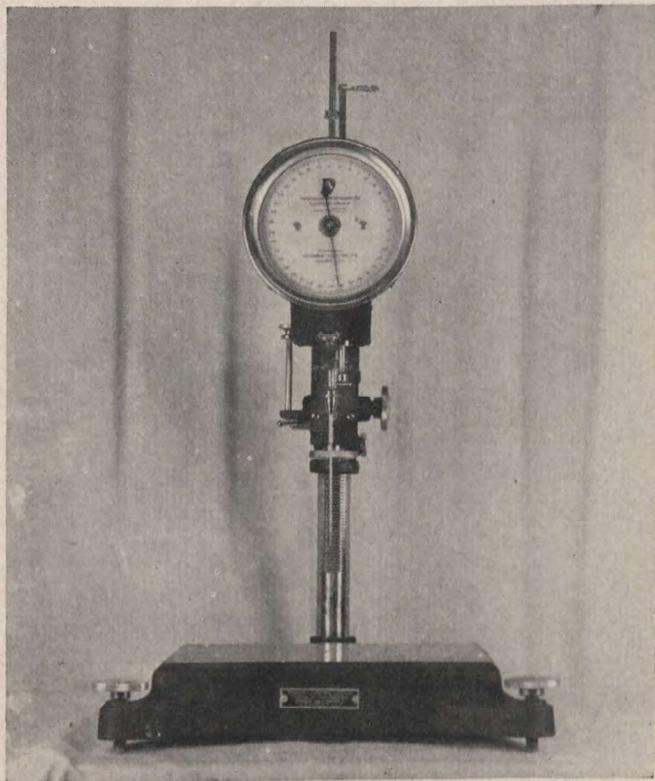


Fig. 3.—Penetrometer modified to measure firmness of texture of recrystallized honey.

The honey used in this test was an Ontario white honey having an original L/D ratio of 1.28 : 1.0. By adding appropriate amounts of dextrose two new "honeys" having L/D ratios of 1.14 : 1.0 and 1.0 : 1.0 were obtained. The moisture content of samples of each of three honeys was adjusted in 1 per cent increments from 15 to 20 per cent.

All the honey samples thus obtained were processed for recrystallization by a standard method and packed in 4-ounce jars for subsequent testing. After storage for one week at 57° F., to allow crystallization to become complete, the jars were placed on the laboratory shelves (65° F. – 76° F.) in simulation of retail storage. Penetrometer readings were taken immediately and thereafter at monthly intervals. In Table 6 are shown the penetrometer readings for each honey at the dates indicated.

¹ G. H. Austin.

TABLE 6.—THE EFFECT OF STORAGE ON TEXTURE OF RECRYSTALLIZED HONEY AS MEASURED BY PENETROMETER

Sample No.	Penetrometer Readings* in mm. at dates indicated					
	Sept. 15	Sept. 22	Oct. 22	Nov. 22	Dec. 22	Jan. 22
A16.....	11.2	11.2	9.7	8.0	8.1	8.6
A17.....	10.4	11.0	11.2	10.8	24.1	30.2
A18.....	11.5	13.8	13.8	17.7	34.8	45.0 ^a
A19.....	12.0	20.9	20.9	45.0	45.0 ^a	45.0 ^a
A20.....	12.2	22.5	22.5	45.0 ^a	45.0 ^a	45.0 ^a
B16.....	6.5	7.2	5.4	5.3	5.9	6.6
B17.....	5.5	6.0	4.9	4.7	6.3	7.0
B18.....	5.8	7.8	6.9	8.6	11.0	11.0
B19.....	6.2	8.6	9.4	13.0	14.9	22.5
B20.....	7.5	10.5	15.5	18.0	35.8	43.8
C16.....	2.6	5.7	5.1	5.8	5.9	6.0
C17.....	2.8	5.3	4.8	5.2	5.9	5.7
C18.....	3.2	5.2	5.2	5.5	7.7	7.6
C19.....	3.4	5.9	7.7	7.9	9.4	9.7
C20.....	3.8	7.9	12.0	13.9	22.0	34.7

* Means of five readings on duplicate samples.

^a Partial change of state.

These results show that in order to maintain a firm texture for at least four months, honey that is to be recrystallized should have a moisture content no greater than 18 per cent if its L/D ratio is above 1.14 : 1.0. The moisture content need not be so stringently controlled provided the L/D ratio is lower than the above.

Prevention of Crystal Development in Packing Liquid Honey

While most domestic honey has an inherent tendency to crystallize (due to dextrose supersaturation), a growing market for liquid honey necessitated some practical method of maintaining honey in a crystal-free state. Of the several experimental approaches tried, a form of heat treatment proved most promising.

The work was begun in 1950 and a technique for the preparation, treatment, storage, and examination of samples gradually evolved. Briefly the technique is as follows:

- (a) Honey samples are introduced into pyrex tubes (9 mm. by 80 mm.) and heat-sealed.
- (b) Tubes are immersed in a constant temperature water bath for definite periods.
- (c) After treatment samples are stored at 57° F. (optimum temperature for growth of dextrose crystals in honey.)
- (d) At weekly intervals (or as often as conditions warrant) samples are examined under polarized light through 9x magnification.

The 1950 tests covered a range of heat treatments from 120° F. to 200° F., and holding times from five minutes up to five days (for the lower temperature). The treatments involving low temperature and long holding times were not successful. For example, crystals appeared after eight weeks of storage in honey that had been held at 140° F. for four hours. In the treatments where honey was held for short periods at high temperatures crystal development was suppressed for more than six months depending upon the treatment.

Due to the heat-sensitive nature of honey it was necessary to establish minimum "time-temperature" combinations. An alfalfa honey, having a demonstrated rapid crystallizing potential, was selected for the tests. In Table 7 are recorded the treatments and crystal-free periods of samples held in storage at 57° F.

TABLE 7.—TIME-TEMPERATURE TREATMENTS OF ALFALFA HONEY

Sample No.	Treatment		Percentage showing crystals after 2 years
	Temperature °F.	Time (min.)	
1.....	160	5	60
2.....	160	15	0
3.....	160	25	0
4.....	170	5	0*
5.....	170	15	0
6.....	170	25	0
7.....	180	5	0
8.....	180	15	0
9.....	180	25	0
10.....	190	5	0
11.....	200	5	0
12.....	210	5	0

* Selected as minimum "time-temperature" treatment.

By immersing samples of this honey in water at 170° F. for five minutes crystal development has been suppressed for more than two years in storage at 57° F. Apparently all crystals were completely liquefied with few (if any) nuclei left as starting points for re-growth. The success of the treatment is probably due to the fact that only a thin film of honey is exposed and consequently heat penetration is even and rapid.

Extracted honey contains crystals of various sizes—depending upon its floral source. Since the amount of heat required to melt crystals is directly related to their size, further tests were necessary before a generally applicable "time-temperature" recommendation could be made.

In this investigation an alfalfa honey having large crystals was used in one series of treatments. For comparison, the same honey after heating and re-seeding was used in a similar series to provide a finely crystallized honey. Using the standard technique already described, samples of both coarse and fine crystallized honey were heat-treated. The treatments listed in Table 8 show that the size of crystals in honey to be processed is a major determinant in the choice of heat treatment.

TABLE 8.—EFFECT OF CRYSTAL SIZE ON RE-DEVELOPMENT OF CRYSTALS AFTER VARIOUS HEAT TREATMENTS

Crystal type	Treatment		Percentage showing crystals after		
	Temp. °F.	Holding time—min.	6 mo.	9 mo.	12 mo.
Fine.....	160	20	20	20	20
Coarse.....	160	20	80	80	100
Fine.....	170	10	0	0	20
Coarse.....	170	10	60	60	80
Fine.....	170	15	0	0	0
Coarse.....	170	15	0	20	20
Fine.....	180	5	0	0	0
Coarse.....	180	5	20	20	40
Fine.....	180	10	0	0	0
Coarse.....	180	10	0	0	0
Fine.....	190	2	0	0	0
Coarse.....	190	2	40	40	60
Fine.....	190	5	0	0	0
Coarse.....	190	5	0	0	0

Pre-heat Treatment

When naturally crystallized honey is being processed it is necessary to apply a mild heat treatment so as to render it fluid enough for pumping. Obviously the duration of this treatment will have a direct bearing on the size of the crystals in the honey as it enters the process line.

In these tests one batch of honey was held at 120° F. for three hours; the other was held at the same temperature for 18 hours. For the two treatments, the holding time shown in Table 9 is the minimum for each of the temperatures listed that is necessary to suppress crystal development for at least six months.

TABLE 9.—EFFECT OF PRE-HEAT TREATMENT ON SUPPRESSION OF CRYSTAL GROWTH AFTER PROCESSING

Pre-heat 120° F. for	Treatment Temp. °F.	Holding time (min.)
18 hr.....	170	5
3 ".....	170	10
18 hr.....	180	1
3 ".....	180	5
18 hr.....	190	0.5
3 ".....	190	2.0

It is apparent that when honey is pre-heated long enough to remove most of the visible crystals, the holding time at high temperatures can be materially reduced with no loss in effectiveness.

Pure Solutions

While the ratio of levulose to dextrose in honey appears to be the main factor governing its crystallizing behavior, certain minor components seem also to have an effect. In order to evaluate the effect of the L/D ratio without the complications of other factors, solutions of levulose, dextrose, and sucrose were prepared with concentrations and proportions in the same range as those found in honey.

To avoid overheating, dilute sugar mixtures were made up and reduced to the required concentration under vacuum. Thirty-five replicates were prepared for each of three groups which had L/D ratios of 1.30 : 1.0, 1.15 : 1.0, and 1.0 : 1.0 respectively. All samples were stored at 57° F. Periodic examination established the time at which crystals appeared in the various ampules.

To determine the relative crystallizing rates of the three groups it is necessary to consider not only the number of replicates in which crystals appear but also the time lapse to their appearance. Hence, a formula was worked out to yield a "coefficient of crystallization" for each group at certain periods after the samples were prepared. This factor is defined as the product of the percentage of samples in which crystals appear and the reciprocal of time in days to their appearance. The coefficients for successive periods are, of necessity, the cumulative totals for the periods in which crystals appeared. A condensation of the data gathered in these tests is presented in Table 10.

TABLE 10.—CONDENSED DATA FROM PURE SOLUTION STUDIES SHOWING THE CUMULATIVE COEFFICIENTS OF CRYSTALLIZATION FOR EACH TYPE OF SOLUTION

Time in days	L/D = 1.0 : 1.0		L/D = 1.15 : 1.0		L/D = 1.30 : 1.0	
	No. samples crystallized	Coefficient crystallization	No. samples	Coefficient crystallization	No. samples	Coefficient crystallization
51.....	1	0.056	—	—	—	—
62.....	3	0.151	2	0.092	—	—
85.....	6	0.2794	3	0.1279	—	—
133.....	7	0.3053	4	0.1514	2	0.0566
157.....	8	0.3280	7	0.2154	3	0.0759
163.....	9	0.3507	9	0.2592	4	0.0951
191.....	13	0.4385	12	0.3232	5	0.1120
216.....	15	0.4823	13	0.3441	8	0.1623

The relative degree to which the L/D ratio (within the prescribed limits) contributes to the crystallization rate may be found by comparing the coefficients of each group within any one period.

Effect of Addition of Colloidal Material and Changes in pH on Crystallization Rate of Honey

Colloid Studies.—Honey contains various amounts of colloidal material depending upon its floral derivation. To a limited degree viscosity is directly related to the amount of colloidal material present. With increased viscosity the ability of dextrose molecules to orientate into the crystal lattice is impeded. Therefore, it was proposed to add inert colloidal material to liquid honey and to determine its effect on crystallization.

To different samples of the same honey colloidal suspensions of sodium alginate and carageenin were added to yield concentrations of 0.06 and 0.001 per cent by weight. Subsequently samples were treated by our standard technique.

No significant difference in the crystallization rate of honey resulting from added colloidal material could be shown in these tests. In addition, the incorporation of such material imparted to the honey an atypical gel-like consistency. These tests were discontinued.

pH Studies.—The degree of acidity of a honey measurable by pH value readings affects the stability of its colloidal content. Adjustments were made in the acidity of a honey so as to render the colloids unstable (isoelectric point). However, a buffer system apparently exists in honey which tends to cause the pH value to revert to its natural level. Study of this approach to the problem was abandoned.

Low-temperature Storage of Liquid Honey.—The concept that increased viscosity may impede the migration of dextrose molecules to crystal nuclei was tested by another method. In this case the fact that viscosity increases as temperature decreases was studied.

A normally granulated Ontario honey (probably alsike) was heated mildly so that it was clear of visible crystals yet retained crystal nuclei. Samples were drawn off in two-ounce vials and stored at 0° F.; at 32° F.; at 57° F.; and on the laboratory shelves at room temperature.

That the honey contained many crystal nuclei is exemplified by the fact that crystals appeared in samples stored at 57° F. in five weeks and those at room temperature in nine weeks. During this interval no crystals appeared in the honey stored at the lower temperatures so samples were removed and placed in the 57° F. storage. In these samples crystals did not appear for more than three months. It would appear that not only do low temperatures (0° F. and

32° F.) prevent crystal formation in honey during storage but also they have a latent inhibitory effect when honey is removed to ordinary storage temperatures.

When these tests were repeated with an alfalfa honey, low temperature storage seemed to have little or no effect on its subsequent crystallizing behavior.

“Stack Heat” and Cooling Rates of Commercially Packed Honey

“Stack heat” is a common phenomenon in the food packing industry and is the result of “hot” packing the product in commercial cartons and stacking these in solid blocks. Because of the heat-sensitive nature of the product, stack heat may seriously affect the quality of both liquid and recrystallized honey.

The time-lag in cooling of both types of honey was measured by thermocouples under conditions simulating those that prevail in the trade.

In a preliminary test, the cooling rate of liquid honey packed at 120° F., was measured at strategic points in a three-carton stack which was built up in a freezer cabinet held at $10 \pm 2^\circ$ F. Honey at the outside corners of the stack took 11 hours to cool from 120° F. to 60° F. During the same interval (11 hours) the temperature of honey at the center of the stack dropped only 30 degrees (to 90° F.). Individual bottles in the same test cooled to 50° F. in one hour. It is apparent that when honey is stored at room temperature and in much larger stacks (as is the common practice), the time-lag before its mean temperature falls to 80° F. (critical temperature for quality retention) will be considerably extended.

In order to accelerate the dissipation of heat from the stacked honey, a variable speed fan was introduced into the cooling cabinet. The effect of air movement on the cooling rate of individual bottles at various temperatures and air speeds was measured. Some of the data so gathered appear in Table 11.

TABLE 11.—EFFECT OF AIR MOVEMENT ON COOLING RATE OF HONEY IN ONE-POUND JARS

Temp. cabinet	Air speed ft./min.	Initial temp. (honey)	Final temp. (honey)	Cooling time (min.)
28° F.....	800	127° F.	36° F.	170
28.....	940	124	36	170
28.....	1,010	122	36	160
30.....	1,075	119	36	150
-2.....	1,075	120	28	75
8.....	Static	114	20	235

A definite increase in cooling rate results from air movement.

In further tests the influence of shape of the jar (round versus flat) on cooling rate was studied. In flat bottles the time taken to cool through the range 120° F.—80° F. was decreased by 17 per cent.

Reducing Time of Storage at 57° F. for Recrystallized Honey

A practice, implicit in “Dyce-Process” recrystallized honey, is to reduce the temperature of newly seeded honey by storing it in commercial containers at 57° F. Generally, it is necessary to leave the honey in storage for from four to five days to allow crystallization to become complete.

This time-lag in production creates several difficulties. It necessitates a cold room large enough to house five days’ plant production or, alternatively, permits only intermittent operation. Therefore, it was proposed to seek some method of reducing this storage time.

It has been shown that 57° F. is the optimum temperature for crystallizing honey. Consequently, for high quality it is necessary to reduce the tem-

perature to this point as soon as possible after seeding. In order to reduce the time two distinct methods of approach were used.

In the first, advantage was taken of the fact that because of the high viscosity of honey most of the cooling is by direct heat transfer rather than by convection, and local supercooling occurs in most containers. In primary storage at 40°–45° F., when the surface temperature of honey in 2-pound pails fell to 48° F., the temperature at the center of the pail was reduced only to 59° F. On removal to storage at 65° F. (cellar temperature) heat was transferred from the center to the peripheral honey so that the inner temperature actually decreased during the first few hours of storage at the warmer temperatures. Although only three cases were used to build a stack in the secondary storage, the mean temperature of the honey remained below 60° F. for more than 34 hours.

Actually, the honey temperature was reduced to, and remained in the range of 50° to 60° F. for 46 of the first 58 hours after processing. Compared with this, in the normal method of storage at 57° F., the honey temperature is in this range for only 26 of the first 58 hours. In the standard method this extensive time-lag (36 hours) in reducing the processed honey to the proper temperature range may affect its quality.

It is apparent that under practical conditions the much larger stacks available for secondary storage would effectively extend the period in which the honey would remain in the proper temperature range.

Recommended Recrystallization Technique:—

- (a) In primary storage (40° to 50° F.), stack the cartons loosely in tiers.
- (b) Remove the cartons after 24 hours and stack them in solid blocks at warehouse temperature.

As an alternative to the above method of reducing both the total time in ordinary storage and the initial lag in reducing honey temperature to the optimal range, ventilating the cardboard cartons to provide cross-ventilation was tested.

On each of the four sides (but not on the top or bottom) 1¼ inch holes were cut in the carton, (Fig. 4) in line with the free space between the cans, to provide air flow.

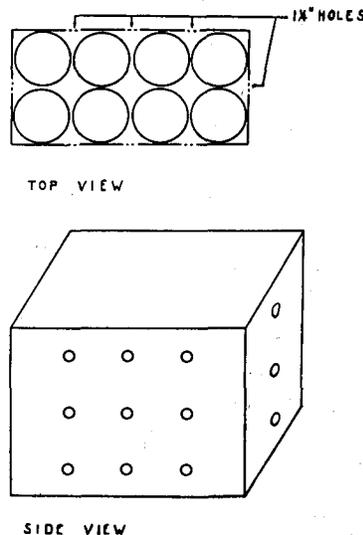


FIG. 4.—Standard carton ventilated to allow air-flow.

Warmed honey (80°–85° F.), in both a case so ventilated and in a standard case, was placed in the cooling cabinet. Temperatures were taken by means of thermocouples inserted to the geometric center of cans at various points within each case. Part of the data from these tests is shown in Table 12.

TABLE 12.—COOLING RATE OF HONEY IN STANDARD AND VENTILATED CASES AT VARIOUS CABINET TEMPERATURES

Trial No. 1	Temp. (°F.) during storage after—				
	0 hr.	2 hr.	3.75 hr.	7 hr.	9 hr.
Standard case.....	88	77	71	62	59*
Ventilated case.....	87	66	56*	46	42
Cabinet temperature.....	42.5	41	41	41	41
Trial No. 2	0 hr.	2 hr.	5 hr.	7 hr.	24 hr.
Standard case.....	88	82	74	71	58*
Ventilated case.....	88	73	64	58*	55
Cabinet temperature.....	54	55	55	55	55

* Mean temperature of honey in the case has fallen to the optimum range for crystallization.

Results of the test show that by ventilation, primary storage may be reduced from 12 to 4 hours in sub-optimal temperatures. On removal to secondary storage ventilation would be a disadvantage. This can be overcome by stacking the cases tightly with the vents offset from each other to eliminate air-flow.

Where it is desirable, because of equipment already installed, to use 57° F. as the primary storage temperature, the venting of cartons reduces the time-lag in lowering honey temperatures to the desired range from 24 to approximately 7 hours.

Determination of Flow Rates and Viscosity of Honey

The high viscosity of honey at normal temperatures is the most important factor to be considered in the design of extracting and processing equipment. The relationship of moisture content, temperature, and viscosity was determined by two independent methods.

Flowmeter Tests.—A honey "flowmeter", designed at this Division, was used to measure the relative rates of flow of honeys of different densities at fixed temperatures.

The moisture content of samples of an average minimum white honey was adjusted through the range 15 to 20 per cent in 1 per cent increments. Samples were tested in the flowmeter through the normal range of temperatures (70° to 140° F.). In Table 13 the data thus gathered are presented.

TABLE 13.—RELATIVE RATES OF FLOW* OF HONEY OF VARYING DENSITIES AT DIFFERENT TEMPERATURES

Temperature °F.	Percentage moisture					
	15.0	15.9	17.0	17.9	19.0	19.8
70.....	662	364	141	86	44	39
80.....	257	143	69	43	27	20
90.....	101	66	43	25	18	11
100.....	55	41	23	14	10	7
110.....	29	20	13	8	6	4
120.....	19	12	9	5	4	3
130.....	10	6	5	3	3	—
140.....	7	4	3	—	—	—

* Time in seconds for a unit volume of honey to flow through a standard orifice at the temperature indicated.

The influence of temperature and moisture content on the rate of flow of honey is readily seen. For example, a 19 per cent honey flows five times as rapidly as a 15 per cent honey at 100° F. Similarly, a 17 per cent honey flows more than twice as fast at 120° F. as at 100° F. and three times as fast at this temperature as at 80° F.

Since such familiar honey-house operations as extracting, pumping, and settling are all dependent upon flow-rate, the relationship of the tabulated flow times for each honey, over the range of temperatures, may be taken as a criterion of operational efficiency.

Kinematic viscosity tests.—A more precise measurement of honey viscosity at various temperatures and densities was conducted to facilitate the designing of new processing equipment. The kinematic viscosity of honey was measured (in centistokes) with an Ostwald-Fenske viscometer. With this method of measurement, changes in specific gravity with temperature are disregarded since they are corrected for by a factor which is worked out from the instrument's calibration curve. The moisture contents of samples of white, golden, and buckwheat honey were adjusted as in the previous test. Measurements were made through the range 70° to 190° F. The logarithm of viscosity was plotted against temperature on a semi-log graph. From the linear plot the values at fixed temperatures were interpolated and are tabulated (for the white honey) in Table 14.

TABLE 14.—KINEMATIC VISCOSITY (IN CENTISTOKES) OF A WHITE HONEY AT VARIOUS TEMPERATURES AND DENSITIES

Temperature °F.	Percentage moisture				
	14.6	15.5	16.7	18.1	19.1
80.....	13,180	8,220	5,310	2,978	2,110
90.....	6,494	4,190	2,760	1,585	1,016
100.....	3,320	2,178	1,489	893	665
110.....	1,858	1,245	855	535	407
120.....	1,092	740	524	330	257
130.....	667	458	332	216	171
140.....	449	295	217	146	102
150.....	280	198	149	103	83
160.....	189	143	106	74	61
170.....	135	100	77	56	46
180.....	96	73	58	43	36

Further data on the viscosity of other types of honey will be reported in more detail in another publication.

Equipment for Processing Honey¹

Honey Processing Equipment.—The equipment in current use by the majority of honey packers in Canada is designed to process honey in "batches". Such processing is necessarily intermittent and limits the effective use of labor and supplementary equipment. Consequently, the packing costs are higher than would be the case if the product were processed in a "continuous-flow" system. Moreover, under the present system some deterioration of flavor occurs when honey is not cooled rapidly after being heated to the required pasteurization temperature.

A project was undertaken in 1951 to investigate different types of processing equipment used by other food industries with the object of determining their suitability for honey. In addition to this phase of the project it was considered expedient to design new equipment on a pilot-plant scale that might be suitable for the beekeeper packer.

¹ E. Braun.

Laboratory Model.—Tests with a laboratory model under controlled conditions demonstrated the feasibility of heating and cooling honey in a continuous-flow system. Honey could be heated, in this equipment, to 160° F. with a water temperature of 162° F. In order to maintain an adequate flow-rate when cooling honey to 80° F. it was necessary to hold the temperature of the coolant at not less than 76° F.

Plate-Type Heat Exchanger.—A plate-type heat exchanger, similar to that used in the dairy and other food industries, was modified for tests with honey. A double-jacketed tank was used to pre-heat the honey to 100° F. A positive drive pump forced the honey into the heating section of the pasteurizer. The honey, in a thin layer approximately one-twentieth of an inch in thickness, was pumped between sets of corrugated plates, while hot water was pumped in a counter-flow through the space between alternate plates. The honey heated to 170° F. flowed into a holding tube and passed through the cooling section, where the temperature of the honey was reduced to 80° F. A flow rate of 345 pounds per hour was attainable with the test equipment. The total lapsed time from the pre-heat unit through the heating and cooling unit was six minutes.

Pressures which developed on cooling a honey of high density (15 per cent moisture) exceeded 25 pounds per square inch—the maximum pressure recommended by the manufacturer. A new unit utilizing high pressure plates now being manufactured (but not yet tested) should be satisfactory for a high density honey. This equipment was investigated for the use of large commercial honey packers.

Tubular-Type Heat Exchanger.—A pilot-plant unit, suitable for the small independent honey packer, was constructed in co-operation with the Engineering Section of the Field Husbandry Division. The equipment consisted of three water baths, electrically heated and thermostatically controlled, for pre-heating, pasteurizing, and cooling. A positive drive Pesco fuel pump, similar to those used on tractors, was found to be satisfactory for pumping honey. One-inch (outside diameter) copper tubing was used between the pre-heat tank and the pump and from the pump to the pasteurizing tank. A by-pass, with a mechanical valve, was installed beyond the pump to reduce the pressure which developed in the cooling unit.

A flow-rate of approximately 100 pounds per hour was obtained by heating in a 150-foot length of tubing but when a similar length was used for cooling to 80° F. the flow-rate was reduced to 9 pounds or less per hour.

In order to establish minimal lengths for heating and cooling honey, thermocouples were inserted at various distances in 150-foot lengths of coiled copper tubing ($\frac{1}{4}$ inch outside diameter). It was found that honey could be heated to the desired temperature in 15 feet of tubing and could be cooled in about the same length. Provision for a holding time in the pasteurization unit necessitated the use of 20-foot lengths of tubing, and the same length of tubing was used for cooling. The honey was pre-heated to 100° F., heated to 196° F., and cooled to 57° F. Mechanical stirrers operating at approximately 290 r.p.m. were used to agitate the water in the baths to prevent uneven heating and cooling.

Following these preliminary tests, a unit was constructed in which the heating tubes were 20 feet long, and the five cooling tubes ($\frac{5}{8}$ inches outside diameter) were run from a common header. In this unit honey could be heated and cooled at 92 pounds per hour. This volume was stepped up in a later model which had three $\frac{1}{4}$ -inch tubes for heating and fifteen $\frac{5}{8}$ -inch tubes for cooling.

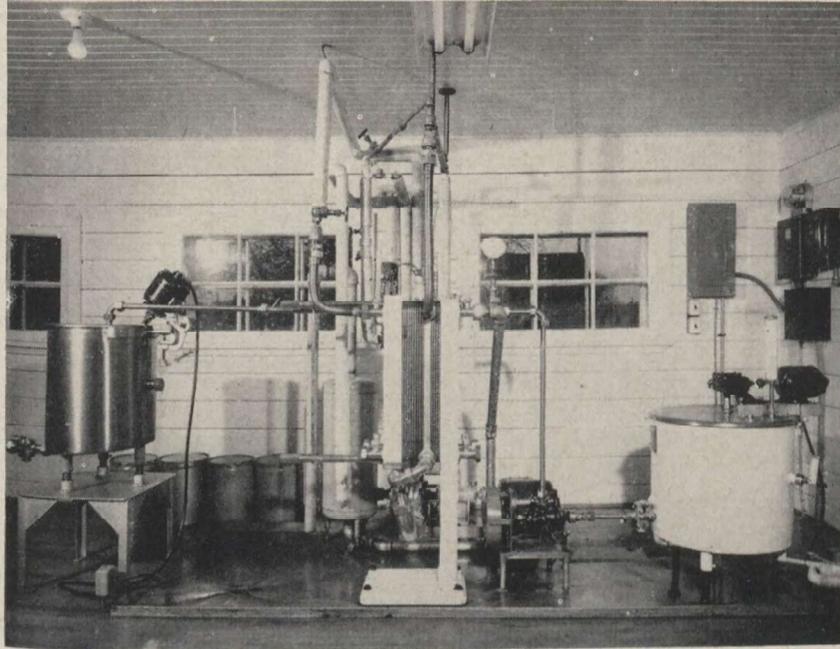


Fig. 5.—Plate-type Heat Exchanger. Right to left: Preheat tank, Moyno pump, heat exchanger and storage tank.

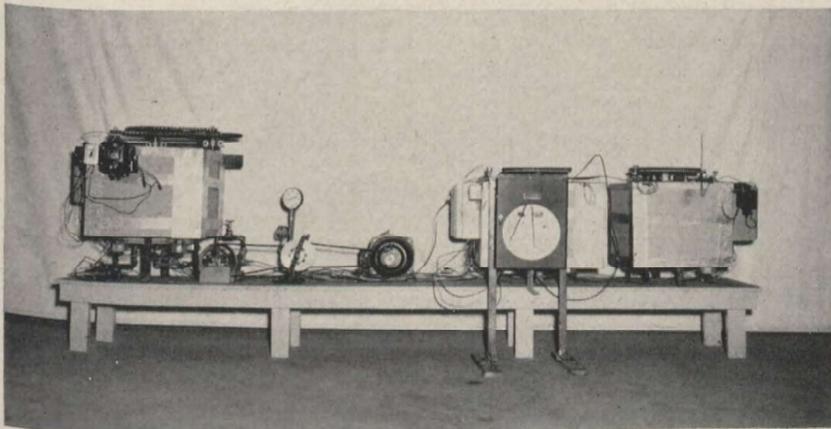


Fig. 6.—Tubular-type Heat Exchanger. Left to Right: Preheat tank, pump and by-pass, pasteurizing and cooling tanks.

Pasteurization of Honey by Irradiation¹

In co-operation with the Bacteriology Division, Science Service, and [the General Electric Company, Schenectady, N.Y., an experiment was conducted to determine the killing effect of high velocity electrons on osmophilic yeasts in honey.

¹ C. A. Jamieson.

Two samples of honey showing yeast counts of 5,900 and 7,400 per gram were forwarded to the General Electric Company for irradiation. A resonance transformer-type cathode-ray unit provided a source of high energy electrons. Two-gram samples of honey, sealed in polyethylene containers, were irradiated from both sides with dosages ranging from 0.25×10^6 to 5.0×10^6 roentgen equivalents. Following treatment the samples of honey were cultured for yeasts at the Bacteriology Division. No yeasts survived dosages of 0.5×10^6 r or higher. Six cultures of osmophilic yeasts, grown on honey agar, were transferred to samples of liquid honey for additional tests.

The irradiated samples were again plated and counted. The results obtained with the inoculated honey are outlined in Table 15:

TABLE 15.—THE KILLING EFFECT OF HIGH VELOCITY ELECTRONS ON A MIXED CULTURE OF OSMOPHILIC YEASTS IN HONEY.

Dosage $\times 10^6$ r	Number of survivors	Percentage survivors
0 (control).....	128,000	100
0.025.....	68,000	53
0.05.....	21,000	16.4
0.075.....	12,000	9.3
0.1.....	9,500	7.4
0.25.....	2,800	0.44
0.5.....	570	.015
1.0.....	20	0
2.0.....	0	0

It is probable that certain strains of yeasts in the inoculated samples of honey were more highly resistant, as complete killing did not occur until a dosage of 2.0×10^6 r was applied.

Sterilization of Contaminated Combs and Honey by Irradiation²

In additional tests hive equipment and honey containing spores of American foulbrood were irradiated by the General Electric Company to determine the killing effect of various dosages of high velocity electrons. Thin sections of comb containing scale material; scale placed on sterile wood covered with a thin layer of beeswax; small sections of whole comb with scale; and liquid honey inoculated with spores from scale were irradiated.

In thin comb sections complete destruction of American foulbrood spores was achieved at an irradiation dose of 1×10^6 r or greater. Scale on wood covered with wax was inactivated by 1 and 2×10^6 r. The section of whole comb was exposed to a high dosage of 5×10^6 r. When this comb was incorporated in a frame and placed in a colony no disease developed. The scale was removed by the bees and the queen laid in the cells, indicating that irradiation imparted no objectionable properties to the combs. The lethal dose for honey containing 4.2 million spores per gram was in the order of 2 to 4×10^6 r. There was a marked reduction in viable spore count at a dose of 0.025×10^6 r, thus indicating that strains vary in their degree of resistance to this treatment as was evident with osmophilic yeasts.

² C. A. Jamieson.

It has been shown that yeasts and American foulbrood spores in honey and contaminated apiary equipment can be sterilized by irradiation with high velocity electrons. It is of interest to note that irradiation is not accompanied by an appreciable increase in temperature and thus it is often referred to as "cold" sterilization. Because of its cost the use of this type of equipment for sterilizing honey or diseased equipment is not practical.

BEE DISEASES

The investigations conducted on the control of American foulbrood, sacbrood, *Nosema* disease, and other related studies were undertaken in co-operation with the Bacteriology Division, Science Service.

Experiments with Antibiotics for Control of Sacbrood Disease

Although this virus disease is not generally serious in an apiary it was evident in several colonies in the experimental apiary in early July, 1949. In view of reports on successful treatment of certain virus diseases with chloromycetin and aureomycin some experiments were conducted to determine their effect on sacbrood. Three colonies of bees were each fed one gallon of 50 per cent sugar syrup inoculated with a heavy suspension of crushed infected larvae. One colony received 200 mgm. of aureomycin, one received 200 mgm. of chloromycetin, and one was left as control. No disease developed in either of the colonies receiving antibiotics but abundant infection occurred in the control. In a second experiment chloromycetin was effective in controlling the virus, but not aureomycin.

A more elaborate experiment was set up with inoculated colonies in 1950 as shown in Table 16.

TABLE 16.—EFFECT OF CHLOROMYCETIN AND AUREOMYCIN ON SACBROOD IN THE APIARY¹

Treatment	Examination after 48 days
Sacbrood inoculum alone.....	+
Sacbrood inoculum after filtration through Seitz filters.....	++
Sacbrood inoculum heated 10 minutes at 80° C.....	-
Inoculum + Chloromycetin, 200 mgm./gal. syrup.....	-
Inoculum + Chloromycetin, 100 mgm./gal. syrup.....	-
Inoculum + aureomycin, 200 mgm./gal. syrup.....	+++
Inoculum + aureomycin, 100 mgm./gal. syrup.....	+

¹ -, no infection.

+ to +++, increasingly severe infection.

Chloromycetin again exerted complete control whereas aureomycin did not. Filtration of the inoculum through sterile Seitz filters permitted the virus to pass. Heating the inoculum to 80° C. apparently inactivated it.

Results of the three separate tests show that chloromycetin may prevent the development of sacbrood in a colony. However, when this antibiotic was fed to a colony badly infected with the disease it did not cure it. This problem requires further investigation.

Studies on the Control of American Foulbrood¹

In 1946 an out-apiary of ten colonies was established for the purpose of determining the effectiveness of sulphathiazole and penicillin in the control of American foulbrood. The colonies were inoculated with the disease by inserting heavily-infected combs into the brood chambers early in the season. When the disease was manifested in new combs in the brood chambers, treatment with medicated syrup was begun. As published in the Progress Report (1937-1948) sulphathiazole showed considerable promise in controlling this brood disease, while penicillin was ineffective. In the fall of 1946 each of the colonies was fed 1 gallon of 60 per cent syrup containing one gram of sulphathiazole. A recurrence of the disease was evident in the early summer of 1947 in five colonies while the remaining five were free of disease. The diseased colonies received feed containing sulphathiazole and by fall appeared to be free of the disease. None of the colonies was fed sulphathiazole in the fall of 1947. No sign of disease was manifest in any of the colonies during 1948. Four of the original ten colonies died during the period 1948-1952 from causes other than disease.

In order to determine if spore material remained in a dormant state within the hives (possibly in cells of unused honey and pollen) the following procedure was adopted:

1. The bees from the original six colonies were shaken on disease-free, dry combs and fed sugar syrup.
2. The honey from each of the hives was extracted and fed to six package colonies installed on dry, disease-free combs.
3. Six 2-pound packages were installed on the brood chambers of the original colonies.

The brood of the eighteen colonies was examined every two weeks but no disease appeared in the colonies throughout the year. From the results of the final test it was apparent that the bees and equipment were free of disease.

While sulphathiazole was shown to be effective in inhibiting the development of the organism causing American foulbrood, sulphadiazine and sodium sulphathiazole were also tested in other experiments. Both of these sulpha drugs showed the same order of activity as sulphathiazole against the disease.

Stability of Sulpha Drugs in Honey

An experiment was initiated in 1949 to determine the stability of certain sulpha drugs in honey. Two grams of each of the following compounds: sulphathiazole, sodium sulphathiazole and sulphadiazine were added to three pails, each containing 20 pounds of honey. Five pounds of medicated honey from each pail was diluted to provide one 10-pound pail of honey syrup to which was added a suspension of spores of *Bacillus larvae*. One scale, which would contain approximately two billion spores, was used for each treatment. Three 2-pound packages were established on dry, disease-free combs and fed the medicated-honey-inoculum syrup. A fourth package was established and fed a honey syrup containing inoculum as a check. The package colonies were fed the materials during a period when there was a dearth of floral nectar. The remaining lots of honey were stored in a warm room (85° F. ± 8° F.). The same procedure was followed in subsequent years. The results of this experiment are presented in Table 17.

¹ C. A. Jamieson.

TABLE 17.—COLONY TESTS ON THE STABILITY OF SULPHA COMPOUNDS IN HONEY

Colony	Treatment	Year			
		1949	1950	1951	1952
A	Sulphathiazole (0.5 gm.).....	—	—	—	—
B	Sodium Sulphathiazole (0.5 gm.).....	—	—	—	—
C	Sulphadiazine (0.5 gm.).....	—	—	—	—
D	Check.....	+++	+++	+++	+++

—, negative.
+, heavy infection.

The table above shows that for a period of 3 years no disease developed in the colonies fed drugs that had been dispersed in honey. It is evident therefore, that honey does not effect a breakdown of the sulpha compounds.

Apiary Tests With Antibacterial Agents¹

A number of antibacterial agents (drugs and antibiotics) were tested against American foulbrood in the apiary during the period 1948 to 1953. Pure compounds or concentrates of these were fed in 1 gallon of sugar syrup as described previously in this report. The colonies were examined periodically throughout the summer. The criterion used for evaluating the compounds was complete freedom from infection. The compounds tested, their rate of application, and results are shown in Table 18:

TABLE 18.—EFFECT OF ANTIBIOTICS AND DRUGS ON AMERICAN FOULBROOD IN THE APIARY*

Antibacterial agent	Rate fed	Examination of colonies (days)		
		16 days	32 days	78 days
Penicillin (Procaïne).....	300,000 units	+	+++	
Penicillin (Procaïne).....	600,000 units	+	+++	
Clavacin.....	0.5 gram	+++	+++	
Bacitracin.....	0.5 gram	+++	+++	
Subtilin.....	0.5 gram	+++	+++	
Subtilin.....	5.0 grams	++	+++	
Aureomycin.....	0.004 gram	+++	+++	
Aureomycin.....	0.00045 gram	+++	+++	
Aureomycin.....	0.25 gram	+	+	+
Aureomycin.....	0.5 gram	—	—	+
Hyamine (1822).....	0.5 gram	+++	+++	
Chloromycetin.....	0.5 gram	++	+++	
Terramycin.....	0.25 gram	—	—	—
Neomycin.....	0.5 gram	—	—	+
Magnamycin.....	0.5 gram	+++	+++	—
"Terracon".....	0.5 gram	—	—	—
Sulphamethazine (Sulmet).....	0.5 gram	—	—	—
Sulphadiazine.....	0.5 gram	—	—	—
Sodium Sulphathiazole.....	0.5 gram	—	—	—
Sulphathiazole.....	0.5 gram	—	—	—
Controls.....		+++	+++	

* —, no disease.
+, a few infected cells.
++, appreciable number of cells.
+++, many infected cells.

¹ C. A. Jamieson.

The sulpha compounds, namely, sulphathiazole, sodium sulphathiazole, sulphadiazine, sulphamethazine and the antibiotic, terramycin, along with its impure concentrate "Terracon", prevented the development of the disease. The other materials tested did not show activity against this organism.

Susceptibility of Queen and Worker Larvae to American Foulbrood¹

Worker larvae of the honeybee are most susceptible to American foulbrood during the first day of larval life after which the degree of susceptibility decreases until the third day after hatching. As this period of susceptibility closely corresponds with the period of feeding larvae with royal jelly it was considered expedient to determine the age of susceptibility of queen larvae which are fed this substance throughout their larval period.

Brood combs were placed in nuclei for a period of six hours after which the combs containing eggs were either left in dequeened nuclei or removed and placed above a queen excluder in normal colonies for three days. At six-hour intervals thereafter combs were removed and the larvae sprayed with a suspension of AFB spores so that the brood area of each comb received about one billion spores. The combs were returned to queenless nuclei or to normal colonies above a queen excluder. The bees removed a considerable number of the larvae; consequently, when the remaining cells were capped, the combs were placed in an incubator. Each cell was examined microscopically and cultured.

In the studies with queen larvae both spraying with a spore solution and inoculation of individual larvae of selected age were attempted. Many of the larvae of both treatments were removed by the bees. The bars of treated cells when capped were held in an incubator until the date of emergence.

The results of these experiments are presented in Tables 19 and 20:

TABLE 19.—SUSCEPTIBILITY OF HONEYBEE LARVAE TO AMERICAN FOULBROOD

Age of larvae (hours)	Number of capped cells	Number of diseased larvae
0-6	20 (2 queens)	2 queens
6-12	10 (1 drone)	1 drone, 1 worker
12-18	12	2 workers
12-24	68 (5 queens)	3 queens, 15 workers
24-30	1 (queen)	1 queen
30-36	3 (3 queens)	1 queen
36-42	10 (7 queens)	4 queens, 3 workers
42-48	36 (3 queens)	3 queens, 15 workers
48-54	56 (6 queens)	3 queens, 25 workers
18-24 (check)	45	43
48-54	111	6
54-60	334	0
60-66	91	0
66-108	634	0
at 6-hour increments		

Of the worker larvae sprayed with AFB spores at six-hour intervals no disease developed in those older than 54 hours.

Several queen cells were developed in the nuclei thus providing a means of comparing age of susceptibility to AFB of queen and worker larvae.

¹ C. A. Jamieson.

TABLE 20.—SUSCEPTIBILITY OF QUEEN LARVAE TO AMERICAN FOULBROOD

Larvae age (hours)	Sprayed Larvae	
	Capped cells remaining (out of 12)	Number of diseased Larvae
0-24.....	0	—
24-42.....	0	—
42-60.....	2	0
60-78.....	5	1
78-96.....	5	0
	Individual inoculated larvae	
18-30.....	0	—
48-60.....	4	3
60-72.....	10	1
72-84.....	6	0
84-96.....	10	0
96-108.....	12	0

As compared with worker larvae which were not infected beyond the age of 54 hours, three out of four queen larvae (48 to 60 hours) and one out of ten (60 to 72 hours) were infected.

It would appear, therefore, that continuous feeding on royal jelly has no appreciable influence on susceptibility of larvae to American foulbrood.

Studies on the Chemotherapeutic Control of *Nosema Apis*¹

This project was initiated in 1950 when several antibiotics and drugs were fed to bees in cages to determine their toxicity at certain levels prior to testing their activity against the *Nosema* organism. The following agents were fed to caged bees in 1950: atabrine, paludrine, carbarsone, aureomycin, sulphametazine, emetine, ceepryn, diodoquin, chiniofon, diiodo-OH, iodo-Cl-OH. All the above compounds were toxic at the concentrations used with the exception of sulphametazine and diodoquin. The above compounds were fed to bees in a 60 per cent sucrose solution containing 30 to 50 million *Nosema* cysts per cage. The amount of each substance used was slightly lower than that shown to be toxic. The death rate of the bees with some of the materials exceeded that of the inoculated control, indicating that the concentrations were above the level of safety. The experiment was repeated with certain of the compounds together with chloromycetin and sulphaquinoxaline. Only sulphaquinoxaline appeared to exhibit an appreciable therapeutic effect against the *Nosema* organism.

Early in 1951 a new antibiotic, fumagillin, which was reported to have a marked amoebicidal action, was obtained for testing against *Nosema apis*. The antibiotic was dissolved in methyl alcohol and diluted to a definite volume. One millilitre of inoculum, consisting of 35 million cysts of the organism per ml., together with the required dosage, was added to a sucrose solution. The results of the first experiments with this antibiotic were promising and a second experiment was conducted with certain modifications. The results of both experiments are summarized in Table 21.

¹ C. A. Jamieson.

TABLE 21.—EFFECT OF FUMAGILLIN ON *NOSEMA* DISEASE OF ADULT HONEYBEES

Treatments	Percentage dead bees with <i>Nosema</i> infection
	(17-day period)
EXPERIMENT I	
1. Uninoculated.....	0
2. Inoculated.....	76
3. Inoculated + fumagillin (0.15 mgm./30 ml.).....	38
4. Inoculated + fumagillin (0.75 mgm./30 ml.).....	18
EXPERIMENT II	
1. Uninoculated.....	0
2. Inoculated.....	76
3. Inoculated + solvent for fumagillin in amount used for No. 5.....	73
4. Inoculated + fumagillin (0.5 mgm./30 ml.).....	6
5. Inoculated + fumagillin (1.0 mgm./30 ml.).....	2
6. Same as No. 5 but held 2 days before feeding; cysts then centrifuged, washed, and resuspended in sugar syrup.....	62

It is clear from the results summarized in Table 21 that fumagillin caused a significant reduction in the number of bees infected with the disease and that this inhibition was not due to the action of the solvent. Moreover, it would appear that the cysts themselves are not affected by the antibiotic (compare treatments 5 and 6) but that this compound probably exerts its effects when the cysts germinate. A marked decrease in infection occurred with an increase in the dosage of the antibiotic.

Two other antibiotics namely, thiolutin and magnomycin, were tested against the *Nosema* organism in 1953. Both of these materials proved to be ineffective in inhibiting the development of the organism in bees housed in cages.

Studies on the Control of *Nosema* Disease in Colonies¹

Package Colonies.—In the spring of 1952 an experiment was undertaken to evaluate the effectiveness of fumagillin in controlling *Nosema* disease in package colonies. Seventy-eight, 2-pound packages were shaken from colonies at an out-apiary on May 26. The packages were stored in a cellar for five days to provide a holding time comparable to the time required to ship packages from the southern United States.

The packages were divided into six groups of thirteen colonies, which were treated as follows:

Group	Feeding Treatment
A	Control—3 feedings of sugar syrup.
B	Control—Inoculum + fumagillin @ 17.5 mgm./col. (2 feedings in syrup + 1 feeding of syrup only)
C	Inoculum + fumagillin @ 17.5 mgm./col. (2 feedings in syrup + 1 feeding of syrup only)
D	Inoculum + fumagillin @ 17.5 mgm./col. (3 feedings in syrup)
E	Inoculum + fumagillin @ 35.0 mgm./col. (2 feedings in syrup + 1 feeding of syrup only)
F	Inoculum Control—(2 feedings of syrup only + 1 feeding in syrup)

¹ C. A. Jamieson.

The inoculum was obtained by removing cysts of the *Nosema* organism from heavily infected bees. This material was fed at the rate of $2,450 \times 10^6$ cysts per colony. The antibiotic (dissolved in methyl alcohol) and the inoculum were thoroughly dispersed in sugar syrup and fed to the colonies when installed in hive bodies. Subsequent feeding treatments were applied at weekly intervals.

A screened cage, with an opening at the entrance and open at the top as well, was fastened to each colony. Bees unable to fly, in a weakened and dying state, were collected from the cages for microscopical examination. Samples consisting of twenty-five bees each were used as a basis for determining the degree of infection. The percentage infection of colonies by groups is shown in Table 22:

TABLE 22.—PERCENTAGE INFECTION —
JUNE 20 (AVERAGE OF COLONIES IN
EACH GROUP)

Group	Percentage infection
A	25.3
B	11.6
C	10.1
D	14.0
E	12.2
F	28.5

Groups A and F showed a higher average infection than other groups.

Several colonies in each group lost their queens and were removed from the experiment.

The brood areas of all colonies were measured (visually) at three periods. The development of the colonies, as measured by the area of comb space occupied by eggs, larvae, and capped cells, is presented in Table 23:

TABLE 23.—AREA OF BROOD (IN SQUARE INCHES) AT THREE PERIODS (AVERAGE OF
COLONIES IN EACH GROUP)

Groups	June 18	July 12	August 1
A	271	501	733
B	377	862	1,271
C	356	892	1,432
D	395	1,137	1,584
E	325	755	1,210
F	184	354	604

Groups A and F produced significantly less brood than the other groups.

At the end of the honey-flow period all colonies were killed and the bees weighed. The average weight of bees and yield for the colonies by groups are presented in Table 24:

TABLE 24.—AVERAGE WEIGHT OF BEES AND PRODUCTION OF COLONIES

Group	Bees lb.	Honey lb.
A	3.5	12
B	8.3	32
C	8.5	22
D	8.8	34
E	7.8	23
F	4.3	13

The colonies in groups A and F did not develop normally during the experimental period due to infection of *Nosema apis*. The honey yield of these groups was significantly less than groups B, C, D, and E, which were fed fumagillin to control the disease. The control group (A) appeared to have a relatively high initial infection.

The low production of the colonies in the experiment can be accounted for by the fact that the packages were not installed until May 31. The experiment was deliberately delayed in order that the feeding treatments could be applied during a period of low nectar availability.

The results of this experiment demonstrate the effect of *Nosema* disease on the production of package colonies. Fumagillin arrests the development of the organism and appeared to be more effective when administered in three feedings. Increasing the dosage from 17.5 mgm. to 35 mgm. per colony did not appear to give more effective control, as indicated by the yield data.

Overwintered Colonies.—In the fall of 1951 a group of sixty-five colonies of bees was used in an experiment to evaluate the effectiveness of drugs in inhibiting the development of *Nosema* disease during the winter period. The population weights of the colonies were adjusted to provide 7 pounds of bees to each hive. The colonies were divided into five groups for the following feeding treatments:

A—Control—syrup only

B—Inoculum control

C—Syrup + fumagillin @ 140 mgm./colony

D—Inoculum + fumagillin @ 140 mgm./colony

E—Inoculum + sulphaquinoxaline @ 300 mgm./colony.

The inoculum was obtained from heavily infected bees and was fed at the rate of 9800×10^6 cysts per colony. Methyl alcohol was used as the solvent for the fumagillin at the rate of one millilitre per two milligrams of the antibiotic. Sulphaquinoxaline was not soluble in water or alcohol and consequently a suspension of the drug was prepared. The drugs and inoculum were dispersed in a 60 per cent sucrose syrup and fed at the rate of 1 gallon per colony.

The colonies, in single brood chambers, were transferred to double-walled cases for wintering. On the first general examination in late March it was evident that many colonies had died. A detailed examination was conducted on April 9. The condition of the colonies by groups is shown in Table 25:

TABLE 25.—SPRING STRENGTH OF COLONIES BY TREATMENTS

Treatment	Average number frames of bees
A—Control.....	5.6
B—Control—inoculum.....	all dead
C—Fumagillin—control.....	5.1
D—Fumagillin + inoculum.....	11 colonies averaged one frame bees—two dead
E—Sulphaquinoxaline + inoculum.....	all dead

From the results shown in Table 25 it would appear that there was insufficient fumagillin fed to group D to inhibit the development of the organism. Although eleven of these colonies did survive they were severely weakened. In the light of subsequent investigations on the stability of fumagillin it is likely that at least part of the antibiotic was inactivated in the sugar solution leaving the cysts of the organism free to develop.

Sulphaquinoxaline, which showed some promise in cage tests, proved to be ineffective in controlling *Nosema* in colonies.

Transmission of Nosema Disease by Infected Brood Chambers.—The brood chambers of colonies inoculated in the fall of 1952 with cysts of the *Nosema* organism were heavily spotted with fecal matter. Several samples of this material were examined and found to contain cysts of the organism. Seventeen of these hive bodies were moved to another apiary and used as brood chambers for package colonies. Packages were installed on May 22 and on May 29 nine of the colonies were fed fumagillin in syrup. The remaining eight colonies were fed syrup only.

On June 12 a sample of twenty-five bees (pollen-collectors) was captured at the entrance of each colony. Microscopical examinations of the bees showed that the group treated with fumagillin averaged 5 per cent infection while the check group averaged 22 per cent infection. No surplus honey was produced by colonies of either group because of poor honey-flow conditions. The results indicate a possible danger of spreading the disease through the use of brood chambers showing dysentery spottings. Such hive bodies should either be used on exceptionally populous colonies or on the colonies treated with fumagillin to control the disease.

Another experiment was undertaken in the fall of 1952 to control *Nosema* disease in overwintered colonies. A group of seventy-two colonies was selected for this experiment. The weight of each colony was adjusted to 8 pounds of bees. The colonies were divided into four groups for the following treatments:

- Group A—Control—syrup only
- Group B—inoculum¹ + syrup
- Group C—Fumagillin² + syrup.
- Group D—Fumagillin + inoculum

¹ Inoculum fed at rate of 175,000 cysts per bee or 14×10^6 /col.

² Fumagillin fed at rate of 0.005 mgm./bee or 140 mgm./col.

The inoculum was prepared by removing cysts from heavily infected bees and dispersing the material in a 60 per cent sucrose syrup. The antibiotic was dissolved in methyl alcohol and dispersed in sugar syrup for feeding the colonies in groups C and D. Each colony received an 8-pound pail of sugar syrup plus the material indicated in the feeding plan.

A rectangular screen cage (described previously in this report) was placed at the entrance of each colony so that weak and dying bees could be collected, as shown in Table 26:

TABLE 26.—DEAD BEES COLLECTED IN CAGES DURING FALL

Group	Average of 18 colonies per group				
	Collection dates				
	Nov. 10	Nov. 18	Nov. 24	Dec. 4	Dec. 12
A—Control.....	54	72	136	72	125
B—Inoculum.....	74	134	237	723	1,134
C—Control + F.....	68	104	171	64	97
D—Inoculum + F.....	68	81	142	92	125

The mortality of the inoculated colonies showed a marked increase from the second collection date and reached an average of approximately one-third of a pound per colony during the last week of the observation period. On the other hand the colonies of group "D", which were fed the antibiotic along with the inoculum exhibited no abnormal death rate.

Samples of dead bees (twenty-five bees per sample) were examined microscopically for the presence of cysts of the organism. The results of the diagnosis for disease are presented in Table 27:

TABLE 27.—PERCENTAGE INFECTION OF COLONIES BY GROUPS

Group	Sampling dates				
	Nov. 10	Nov. 13	Nov. 24	Dec. 4	Dec. 12
A	31	41	28	80	85
B	90	100	98	100	100
C	7	7	11	1	4
D	9	9	7	3	3

The bees in the inoculated group were heavily infected from November 10 onward while the disease appeared to build up in the control group. In both the fumagillin-fed groups the percentage of infection remained low.

In the early spring (March 16) all colonies were examined and those in group "B" were dead. The remaining colonies were examined in detail on April 13 and found to be in good condition. An 8-pound pail of syrup and fumagillin was given to colonies in group C and D, and syrup only to group A. A second feeding was administered on May 28.

Manipulation of colonies in the three remaining groups was similar during the summer season. The honey flow was poor in the district and consequently only a small crop was produced. The average yield of colonies by groups was as follows:

Group A—Control.....	38 lb.
Group C—Fumagillin.....	34 lb.
Group D—Fumagillin + inoculum.....	36 lb.

The difference in production between the groups was not significant.

The results of this experiment show that fumagillin is effective in controlling *Nosema* in established colonies. It would appear, however, that the development of colonies may not be seriously affected by the disease, as indicated by group "A", colonies of which showed a high rate of infection (80 to 85 per cent) in December. Although the inoculated colonies were unable to survive the heavy infection it is unlikely that under natural conditions, colonies would be exposed to a concentration of cysts of the order used in this experiment.

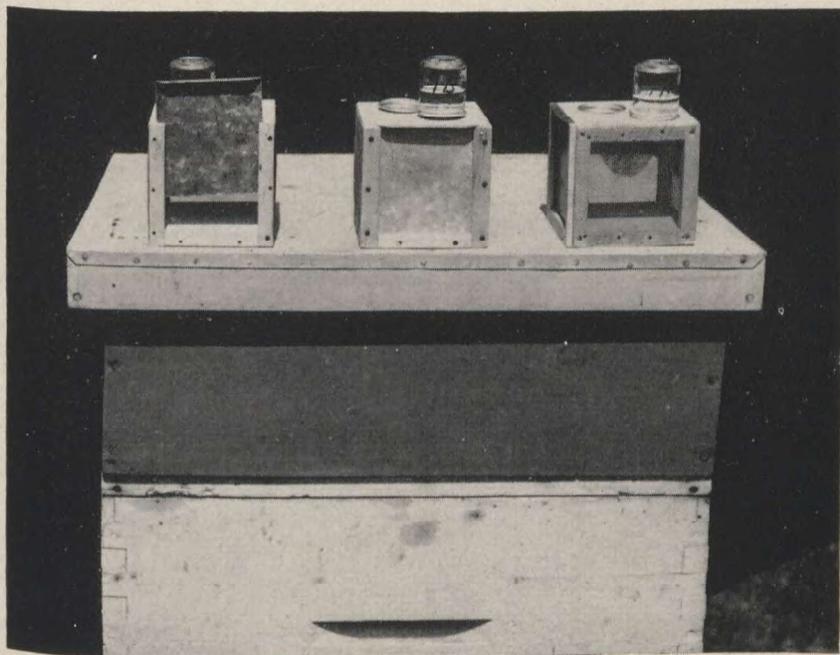


FIG. 7.--Cages used to hold bees when testing antibiotics and drugs against *Nosema* disease.

Stability of Fumagillin¹

In testing the antibiotic, fumagillin, against *Nosema apis* it was indicated that the drug gradually loses its effectiveness when fed in sugar solution to bees. In 1952 a technique was evolved to measure the loss of activity of fumagillin in syrups stored by bees.

¹ G. H. Austin.

Aliquots of fumagillin were dissolved in ethyl alcohol and dispersed in sucrose and honey syrups. These were fed to two nuclei of bees installed on dry comb at a time when little or no floral nectar was available. After the syrups were stored in combs, these were removed to incubation in the laboratory at 88 to 90° F. Samples of syrup were drawn immediately and at two-day intervals, and the fumagillin activity determined by a spectrophotometric method.

Under the test conditions the activity of fumagillin decreased gradually over the test period (57 days). After 36 days the active component had decreased to one-half its original value (i.e., half-life fumagillin = 36 days). There appeared to be little difference in degradation rate in the two syrups (honey and sucrose).

In the other tests it was shown that fumagillin may be fed to bees in concentrations up to ten times the minimum control dosage without undue toxicity. Therefore, in order to compensate for its rate of decay, fumagillin could be fed (particularly for winter feeding) in such concentrations as will provide the minimum dosage when the last of the syrup is used up.

Some Affects on Bee Behavior of Carbon Dioxide Anaesthesia¹

It has been shown that bees follow a regular sequence of hive and field duties which is determined by their age. Following anaesthetization with certain materials this characteristic behavior is frequently disrupted. A study was conducted to determine the age at which bees begin foraging following their anaesthetization as instars or as newly-emerged bees.

Mature larvae, six-day-old pupae, mature pupae, and newly-emerged bees were anaesthetized with carbon dioxide as indicated in Table 28.

TABLE 28.—DATE OF ANAESTHETIZATION AND EMERGENCE OF CO₂ ANAESTHETIZED BEES

Age at treatment	Date exposed to CO ₂	Date of Emergence and marking	Color marked
Mature larvae (uncapped).....	June 7	June 19	White
6-day-old pupae.....	June 13	June 19	Green
Mature pupae.....	June 18	June 19	Yellow
Newly-emerged bees.....	June 19	June 19	Red
Control (newly-emerged bees).....	—	June 19	Blue

On emergence 200 bees from each group were marked as indicated (Table 28) and placed in a nucleus colony.

Each day during the next three weeks, in two one-hour observation periods, returning marked bees were collected at the entrance and destroyed after the type of food they were gathering had been determined.

In Table 29 are shown the number and percentage of bees in each group that were found to have foraged successfully before the normal 20th day after emergence.

N.B. Fumagillin has now been incorporated in a water soluble salt "Fumidil B", which is much more stable in sugar solutions.

¹ G. H. Austin.

TABLE 29.—PERCENTAGE OF BEES IN EACH GROUP FORAGING BEFORE 20TH DAY

Treated group	No. bees foraging before 20th day	Percentage foraging before 20th day
Newly-emerged bees.....	104	52
Mature pupae.....	80	40
6-day-old pupae.....	39	19
Mature larvae.....	44	22
Control (newly-emerged bees).....	36	18

In a further experiment it was proposed to determine if the foraging force of the colony was effectively increased after carbon dioxide anaesthesia. The amount of brood reared subsequent to treatment and the honey production of the colonies were used as criteria. No significant difference could be found due to the anaesthetization treatment. However, a certain amount of disorganization as manifested by increased drifting, seemed to result from anaesthetization.

Longevity Trials.—The effect of graded dosages of carbon dioxide on the life span of caged bees was tested. The results of this work are shown in Table 30.

TABLE 30.—REDUCTION IN LIFE-SPAN OF CAGED BEES AFTER CO₂ ANAESTHETIZATION

Time of exposure to CO ₂	50 per cent dead after
2 min.	13.5 ± 1.5 days
4 min.	14 ± 1 days
6 min.	13.5 ± 0.5 days
8 min.	14 days
10 min.	13.5 ± 0.5 days
12 min.	14 days
14 min.	12 days
16 min.	13.5 ± 1.5 days
18 min.	12 days
20 min.	12.5 ± 0.5 days
Control	20.5 ± 0.5 days

It appears that CO₂ anaesthetization reduces the life-span of bees under these conditions. However, the duration of exposure within the limits tested seems to be of no importance.

Colony Management¹

Effect of Pollen Supplies on Overwintered Colonies

An experiment was initiated in the fall of 1948 to determine the effect of pollen reserves on the populations of overwintered colonies and upon production the following season. The populations of colonies selected for this experiment were determined and adjusted, where necessary, to contain 8 pound of bees per colony. The bees were installed on brood chambers which were prepared as follows:

Group A—Honey plus two combs of pollen

Group B—Honey only

¹C. A. Jamieson and J. P. Caron.

The colonies in single brood chambers were packed in individual, sectional cases with shavings as insulating material.

In the early spring a second brood chamber containing two frames of pollen and honey was given to each colony in both groups. Management of colonies during the spring and summer was similar for both groups. At the beginning of the honey flow all colonies were demareed for swarm prevention.

The average yield per colony of the two groups for the three-year period 1948 to 1951 is presented in Table 31.

TABLE 31.—AVERAGE YIELD OF COLONIES WITH AND WITHOUT POLLEN RESERVES FOR WINTER

Year	Pollen		No pollen	
	No. of colonies	Yield lb.	No. of colonies	Yield lb.
1949.....	27	134	29	148
1950.....	18	118	17	115
1951.....	24	249	23	249

With the exception of 1949, when the difference between the two groups was 14 pounds, the yield has been similar for both groups. These data indicate that brood rearing during late winter is not essential for maximum honey production. On the other hand removal of combs of pollen from colonies in the fall may not be practical under average commercial practices. The presence of a reserve of pollen would not appear to be of any disadvantage to colony production.

Effect of "Side Packing" on the Survival of Colony Population During the Winter¹

In the fall of 1950 forty colonies were selected for an experiment to determine the effect of insulation upon the surviving populations of colonies during the winter period. The weight of bees in each colony was adjusted to 7 pounds. The colonies were housed in single brood chambers containing combs of honey and the equivalent of one comb of pollen.

Half of the colonies were prepared for winter as follows:

Group A: A shallow super filled with shavings, to a depth of 8 inches, was used for insulation above the brood chamber. No insulation was used on the sides but a single layer of tar paper covered each hive.

Group B: Three inches of shavings were used on the sides and 8 inches on the top. The insulating material was covered with a single layer of tar paper.

A bottom entrance was provided for all colonies. On April 13 the colonies were unpacked and the frames containing brood were removed. The queens were caged and left in their respective colonies. During early May the colonies were destroyed and the weight of the surviving population determined in each colony.

There were significantly fewer bees in Group A, the average weight being 45.2 ounces as compared with 58.3 ounces for Group B. On the basis of this experiment it would appear that insulation on the sides of a hive is a factor in the conservation of the populations of colonies during the winter period.

¹ C. A. Jamieson and J. P. Caron.

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 Analyses

Colony Management

Wintering
 Apiary Management of Package Colonies
 Races of Bees for Honey Production

Hybrid Queen Stock Testing**The Foraging Behavior of Honeybees****Pollination**

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